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Actin-dependent endosomal receptor recycling

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Abstract

Endosomes constitute major sorting compartments within the cell. There, a myriad of transmembrane proteins (cargoes) are delivered to the lysosome for degradation or retrieved from this fate and recycled through tubulo-vesicular transport carriers to different cellular destinations. Retrieval and recycling are orchestrated by multi-protein assemblies that include retromer and retriever, sorting nexins, and the Arp2/3 activating WASH complex. Fine-tuned control of actin polymerization on endosomes is fundamental for the retrieval and recycling of cargoes. Recent advances in the field have highlighted several roles that actin plays in this process including the binding to cargoes, stabilization of endosomal subdomains, generation of the remodeling forces required for the biogenesis of cargo-enriched transport carriers and short-range motility of the transport carriers.

Highlights

Actin polymerization on endosomes is primarily controlled by the regulation of the WASH complex activity

Endosomal actin restricts the lateral mobility of cargoes and associated cargo-selective machineries to facilitate the process of cargo retrieval and recycling

Branched actin networks support the formation and stabilization of endosomal tubular domains where cargoes are segregated and recycled

Actin polymerization and actin-based motors facilitate the transport of cargo-enriched post endosomal carriers.

Introduction

The endosomal network is a series of intracellular membrane bound compartments that comprise a central trafficking hub for the sorting of integral transmembrane proteins such as nutrient and iron transporters, adhesion molecules and signaling receptors (together termed 'cargoes'). Cargoes chiefly enter the network from the biosynthetic pathway and following endocytosis from the cell surface, a process that is well known to be regulated by the actin cytoskeleton [1]. The endosomes that first receive cargo following endocytosis are termed early endosomes [2], which undergo transition into late endosomes through a complex alteration in endosomal characteristics that is termed 'endosomal maturation' [2]. Within early and late endosomes cargoes are sorted to one of two fates [3]: either they are sorted for degradation in the lysosome by the endosomal sorting complex required for transport (ESCRT) complex, or they are retrieved from this fate for subsequent recycling back to the cell surface [4], or to the *trans* Golgi network (TGN) [5] (**Figure 1**). The retrieval and recycling of cargoes can occur through a "bulk" flow, as in the case of the Transferrin receptor (TfnR) or through a regulated, sequence-dependent process [6]. The latter is thought to be a multistep process: I) the cargo is first recognised by a retrieval complex(es) and segregated away from the degradative pathway [3,7], and II) the retrieved cargo is then packaged into tubulo-vesicular transport carriers that pinch off from the endosome and couple to cytoskeletal motor proteins for transport to the target compartment [3,7].

This retrieval and recycling process relies on precise sequence motifs in the cytosolic domain of cargo which are recognized by a series of evolutionarily conserved complexes, including the evolutionarily conserved retromer complex [3]. The retromer complex is a trimeric assembly consisting of the subunits VPS35, VPS29 and VPS26 that, directly or indirectly, through the association with sorting nexin proteins, interacts with the retrieval and recycling motifs of cargo proteins [3]. The retromer complex directly associates with sorting nexin 27 (SNX27) that binds the cytosolic domain of transmembrane proteins containing a carboxy-terminal class I PDZ-binding motif, such as the β 2 adrenergic receptor (β 2AR), via its PDZ domain [8-10]. Retromer can also directly interact with sorting nexin 3 (SNX3) [11,12] resulting in the presentation of a binding site for the recognition of a \emptyset X(L/M/V) motif (where \emptyset is an aromatic residue) present in several receptors including the divalent

metal transporter DMT1-II [13] (**Figure 2**). Two other multiprotein complexes, the COMMD/CCDC22/CCDC93 (CCC) complex and the retriever complex, are emerging as important regulators of cargo retrieval and recycling through their association with the sorting nexin 17 (SNX17) that recognizes a NPx(Y/F)/Nxx(Y/F) motif present in cargoes such as β 1 integrin (ITGB1) [7,14-16] (**Figure 2**). Furthermore, it was recently shown that the heterodimers of the Bin/Amphiphysin/Rvs (BAR) domain-containing sorting nexins SNX1/SNX2:SNX5/SNX6, which are responsible for the remodelling of endosomal membranes into tubular profiles, have cargo selective activity and bind a WLM motif in the cation-independent mannose-6-phosphate receptor CI-MPR [17,18] (**Figure 2**).

Actin has long been observed on endosomes [19-24]. In recent years it has become evident that actin regulates several aspects of endosomal biology [25,26], including regulation of endosomal biogenesis and maturation [27-29], endosome morphology [30-32], endosome motility and positioning [33], and sorting of cargo proteins at endosomes [34]. Consistent with this plethora of functions, perturbation of actin dynamics with actin depolymerizing drugs leads to a variety of effects on the endosomal network that include the formation of enlarged and dysfunctional endosomes that are unable to properly sort cargo molecules [32,35-37]. Here, we will review our recent understanding of the pivotal role that actin polymerization and turnover plays in the process of endosomal retrieval and recycling of transmembrane cargoes.

Actin polymerization on endosomes: WASH and cortactin

One of the major actin nucleators on the surface of endosomes is the evolutionarily conserved Wiskott-Aldrich Syndrome protein and SCAR Homolog (WASH) complex [30,31,38]. This is a multimeric assembly of five proteins: WASH1 (WASHC1); Strumpellin (WASHC5); family with sequence similarity 21A and C, FAM21A/C (WASHC2A/C); coiled coil domain containing protein 53, CCDC53 (WASHC3); and the Strumpellin and WASH interacting protein, SWIP (WASHC4) [30,31,39-41]. FAM21 is a critical structural component of the complex that acts as a protein-protein interaction hub while WASH1 is a class I nucleation-promoting factor (NPF), which are activators of Arp2/3-dependent actin polymerization [30,31,39-42] (**Figure 3**).

FAM21 consists of two regions: a “head” domain that assembles with the other components of the WASH complex and an extended (approximately 1110 residues) protruding “tail” domain [43-45]. The FAM21 tail domain contains 21 copies of an LFa motif (consisting of a leucine followed by a phenylalanine and several acidic residues) that can interact with multiple retromer VPS35 subunits [43-45]. The association of WASH to endosomes is partially dependent on its interaction with retromer [43,45,46], with the ESCRT-0 component HRS via a yet to be defined mechanism [47], and possibly, on its inherent ability to bind a broad range of negatively charged lipids [30,40] (**Figure 3**).

The activity of WASH on endosomes is tightly regulated via a process of ubiquitylation and deubiquitylation that precisely tunes actin nucleation. K63-linked polyubiquitylation of WASH1 on its lysine 220 (K220) by the ubiquitin ligase TRIM27 results in its activation leading to enhanced actin-nucleation [48]. TRIM27 is recruited on endosomes through the protein MAGE-L2, which interacts with the retromer subunit VPS35 [48]. At the same time, the complex can “sense” the activation state of WASH and fine tune its activation through a molecular rheostat mechanism which involves the deubiquitylation enzyme USP7 [49]. USP7 interacts with the MAGE-L2-TRIM27 complex to: 1) reduce WASH activity by direct deubiquitylation of the K220 lysine and 2) promote WASH activity by preventing the ubiquitylation of TRIM27 and its degradation [49]. The precise regulation of F-actin deposition is critical for the process of cargo recycling from endosomes as both the overactivation and the inactivation of WASH lead to defects in cargo trafficking [48,49].

A second activator of the Arp2/3 complex that has been described to localise on endosomes is cortactin [35,50-54]. cortactin is a class II NPF that promotes actin assembly by both enhancing Arp2/3-mediated actin polymerisation and by binding and stabilising existing F-actin branches [55]. The targeting of cortactin to endosomal membranes depends on its ability to bind branched actin networks, primarily generated by the WASH complex [30,35], and phosphoinositides, which are an important element of cortactin regulation [54]. In fact phosphatidylinositol 3,5-bisphosphate (PI(3,5)P₂), a phosphoinositide enriched on the cytosolic facing leaflet of the late endosome [56], directly interacts with cortactin via its actin filament-binding region and competes with actin filaments for the binding to cortactin. This suggests that the generation of PI(3,5)P₂ may antagonise cortactin association with

branched actin leading to an inhibition of cortactin-mediated branched actin nucleation and stabilization at the late endosome [54] (**Figure 3**).

Actin in sorting cargoes by restricting their lateral mobility

Intracellular membranes extensively interact, directly or indirectly, with cytoskeletal elements [57]. It is well established that the plasma membrane is pinned by a meshwork of F-actin cortex leading to the formation of membrane nanodomains that exhibit restricted lateral diffusion of their components [57]. The polymerization of branched actin on the surface of peripheral endosomal membrane could stabilize membrane microdomains where newly delivered cargoes are captured in order to prevent their lateral diffusion into the degradative domain [34]. Experimental evidence for this model is largely missing, however there are few observations that point in this direction. First, it was shown that replacement of the retrieval and recycling signal in cytosolic tail region of the β 2AR with an actin-binding domain is sufficient to rescue endosome-to-plasma membrane recycling of the receptor [58]. Furthermore, the analysis of the endosomal sorting of a chimeric TfnR harboring both a non-cleavable ubiquitin moiety and an actin-binding region showed that direct actin binding can overcome ubiquitin-mediated sorting in the ESCRT subdomain [47]. Finally, work in *Dictyostelium discoideum* established that the vacuolar adenosine triphosphatase (V-ATPase) binds with strong avidity to the F-actin network on the maturing lysosomes and that this interaction is necessary and sufficient for the retrieval of the V-ATPase [59]. Taken together these observations suggest that cargo proteins in the endosome membrane can be captured through direct association with the actin meshwork and that this interaction could facilitate their retrieval and recycling.

Actin and WASH in the organization of the retrieval subdomains

The limiting membrane of endosomes is considered to comprise a patchwork of different functional protein-based and lipid-based domains. These include the aforementioned degradative subdomain, enriched with the components of the ESCRT machinery, and the retrieval subdomain, enriched with retromer and other cargo-retrieval complexes [60-62]. The WASH complex is thought to contribute to the

segregation between these two functional opposing subdomains by: i) structuring the retrieval subdomain through interaction with multiple cargo-selective elements and, ii) assembling a meshwork of branched filamentous actin to restrict the lateral mobility of these components and associated cargoes [3] (**Figure 2**). The WASH complex sustains the architecture of the retrieval subdomain by concentrating multiple retromer molecules along the same FAM21 molecule [43,44]. Moreover, the WASH complex interacts with the retromer-associated cargo adaptor SNX27 [8], the retromer-linked SNX-BAR protein SNX1 via RME-8 [63-65], and the retriever complex via the CCC complex [14,66] (**Figure 2**). Recent work suggests that the ESCRT-0 component HRS, which occupies the degradative subdomain, contributes to the recruitment of the WASH complex to the retrieval subdomain to facilitate the recycling of transmembrane proteins [47]. It is likely that this series of interactions contribute to the membrane association of the WASH complex in a cooperative fashion that reflects the density of cargo captured by different retrieval complexes. Concomitantly, the localized nucleation of branched actin could further promote the cohesion of the retrieval domain by restricting the lateral mobility of the cargoes and their associated machineries (see “Actin in sorting cargoes by restricting their lateral mobility”). Consistently, the depolymerization of actin or the depletion of Arp2/3 causes the coalescence of WASH and retromer-positive domains suggesting that the actin branched network is involved in the partition of the discrete retrieval domains [36].

Finally, also actin has a fundamental function in the maturation of the vacuolar domain of the early endosome where the intraluminal vesicles (ILV) are generated, via a process that relies on branched F-actin networks nucleated by Annexin A2, Spire1 and Arp2/3, and containing cortactin and moesin [28,29]. Here, the Annexin A2-dependent regulation of branched actin deposition may contribute to further segregate the degradative domain by separating the recycling tubules away from the maturing multivesicular endosomes [28,29].

Actin in the formation and stabilisation of tubular domains for the recycling of cargoes

The polymerization of F-actin on endosomes is also required for the formation and the stabilization of a subset of tubular profiles, distinct from those mediating bulk flow, that mediate the recycling of the retrieved cargoes to the plasma membrane [52,67]. It has been established that recycled cargo, such as the β 2AR, are recycled via tubular profiles that are characterised by the presence of actin and actin related machineries that include coronin, filamin A, Arp2/3, cortactin and the WASH complex [52,67-70]. The proposed role of F-actin is to stabilise these tubular profiles to allow the entry of cargoes, such as β 2AR, that diffuse more slowly on the endosomal membrane, due to the nature of the retrieval mechanism, and would be therefore excluded from the short-lived sub-set of bulk recycling tubules [52,69]. This actin-based mechanism for the partition of cargoes between the bulk flow pathway and the regulated recycling pathway could conceptually be extended to a multitude of cargoes undergoing sequence-based recycling, including SNX27 cargoes [8,10,71], SNX17 cargoes [14], and SNX1/2:SNX5/6 cargoes [17,18]. Importantly, the Wnt ligand transport protein Wntless (Wls), which undergoes SNX3 and retromer-dependent endosome-to-TGN retrograde transport, was observed to transit through the same tubular domains of β 2AR, implying that actin-rich tubular profiles might act as platform for the recycling of receptors to different cellular destinations [72] (**Figure 4**).

The kinetics of the actin-rich recycling tubular microdomains can be modulated by the phosphorylation of cortactin on the residue Y466 by Src family kinases allowing for signaling-based regulation of the sequence-dependent recycling [73]. Since cortactin can be phosphorylated by a myriad of kinases, such as ACK, FAK, Abl kinase and MEK/ERK [73], it is possible that many signaling pathways might converge to finely regulate the kinetics of receptor recycling under different stimuli. Moreover, filamin A (FLNa), which is an actin-cross-linking protein, localises on the actin-enriched endosomal microdomains where it facilitates the entry of the β 2AR and the chemokine receptor CCR2B into the tubular recycling domains [70]. Importantly, CCR2B and β 2AR signaling induces the phosphorylation of FLNa that promotes receptor recycling [70]. Several other actin regulators play a role in the biogenesis of tubular membrane carriers by regulating actin assembly, disassembly and/or bundling; these include the Cdc42-guanine nucleotide exchange factor FGD6 [74], the regulator of endocytic recycling EHBP-1 [75], and formins [76,77].

Finally, it is emerging that G protein coupled receptors (GPCRs), like β 2AR, can generate sustained signaling from endosomes [78]. This second wave of signaling was shown to be confined to the actin-rich recycling tubular domains and is dependent on the polymerization of an actin network [69], hinting that the regulation of actin-dynamics on endosomal tubular profiles could contribute to the modulation of the signaling cascades.

Actin in regulating the fission of cargo-enriched post-endosomal carriers

The WASH-dependent nucleation of actin also has a primary role in controlling the fission of endosome derived tubules [30,31], including the SNX1/2:SNX5/6-decorated tubules which are responsible for the retrograde transport of the CI-MPR to the TGN [17,18] and several other receptors to the plasma membrane [8,18]. The WASH complex is thought to be recruited on the SNX1/SNX2:SNX5/SNX6 tubular domains via the association with the SNX1 interactor RME-8, also known as DNAJC13 [63-65]. This association provides a means to coordinate the activity of the WASH complex with the membrane remodelling ability of the SNX1/SNX2:SNX5/SNX6 [65]. The WASH-dependent formation of branched F-actin may contribute to the fission of the tubular profiles by providing a pushing force to induce membrane tension [79] (**Figure 4**). Accordingly, depletion of RME-8 or the WASH complex gives rise to long membrane tubules extending from endosomes and prevents the correct retrograde trafficking of the CI-MPR to the TGN [30,31,38,65]. Interestingly, evidence suggests that endoplasmic reticulum (ER)-endosome contact sites regulate WASH-dependent and actin-based severing of tubular elements [80-82] (**Figure 4**). It was shown that a complex between the endosome-localised SNX2 and the ER-localised VAP-A/B mediates the formation of membrane contact sites to regulate endosomal phosphatidylinositol 4-monophosphate (PI4P) microdomains, the perturbation of which leads to exaggerated WASH-dependent actin polymerisation and impairment of retrograde transport [81] (**Figure 3**).

Moreover, the BLOC-1 complex was shown to be required for the elongation and scission of recycling tubules transporting cargoes to the plasma membrane or to melanosomes, which are pigment containing lysosome-related organelles, through

the association with KIF13A and AnnexinA2 [83]. First, BLOC-1 promotes the extension of a nascent tubule along microtubules by interacting with KIF13A, and then it orchestrates the tubule release via Annexin A2-dependent actin polymerization, a process which is WASH-independent [83]. Recently, it was reported that on melanosomes the motor myosin VI couples to the branched actin networks to promote the scission of tubular carriers transporting the SNARE protein VAMP7 [84].

Actin in regulating the transport of cargo-enriched post endosomal carriers

Following the detachment of cargo-laden tubular carriers, actin could also regulate the short-distance motility of cargo-enriched endosomal carriers via two different mechanisms: I) myosin-based transport along existing actin filaments and II) propulsion of endosomes via local polymerization of actin (**Figure 4**). In fact, the class I NPFs N-WASP, which has an established role in regulating actin dynamics during endocytosis, might also localise to early endosomes [26]. N-WASP doesn't seem to play a role in cargo sorting on endosomes as its siRNA depletion had no effect on cargo transport [26]. However, it has been suggested that it could generate an actin comet tail to facilitate the propulsion of endocytic organelles through the cytoplasm [21,52,85]. The WASH complex has been shown to remain on the endosome derived carriers and to participate to the tethering of a sub-population of carriers at the TGN [86]. It remains to be established whether the WASH complex contributes to the short-range motility of endosome derived carriers through a similar mechanism to that of N-WASP.

Moreover, several actin motors play a role in the process of cargo recycling by contributing to the motility of the carriers or by tethering the endosome-to-plasma membrane carriers in to the cortical actin network at the cell periphery [87]. These include myosin-VI, which regulate the tubule formation and the transport of cargo to the endocytic recycling compartment (ERC) [88]; the unconventional myosin-VI MYO6 [89], Myosin-Ib [90], Myosin V, which aids the transport of RAB11-positive vesicles to the cell periphery [91], and Myosin Vb, which is likely to contribute to efficient receptor recycling by capturing and trafficking RAB11-positive vesicles at the cell cortex [92,93].

Conclusions

It is emerging that control of actin dynamics on endosomes is fundamental for the recycling of transmembrane receptors. Actin orchestrates several steps of this process, from the direct capture of cargo which has been delivered to the endosomes, to the generation of the remodeling forces required for the biogenesis of cargo-enriched transport carriers. It has become increasingly clear that the WASH complex and its ability to remodel branched actin networks on endosomes is a key regulator of transmembrane cargo retrieval and recycling. A main challenge in the future will be to distinguish between WASH functions that are the result of its convoluted set of protein-protein interactions and those that depend on its ability to promote actin nucleation. It will also be important to investigate the contribution of the different subunits of WASH complex in the process of cargo retrieval and recycling as growing evidence hints that FAM21 might have functions which are distinct from the other components of the WASH complex in the sorting of endocytic cargo [42]. Moreover, further studies will be required to understand how the spatiotemporal control of actin polymerisation and turnover orchestrates the complex heterogeneity of receptor sorting pathways. Recent evidence seems to suggest that the localised turnover of phospholipids on restricted endosomal subdomains could lead to a localised regulation of the deposition and turnover of actin on endosomes [54,81]. Hence, the interplay between lipid microenvironment and the regulation of actin on endosomes will need to be explored in the future. Finally, we are only now starting to appreciate the role that actin plays in the events that follow the fission of cargo enriched-carriers. Future work will have to address the pending questions about the interplay between actin-based motility and microtubule-based transport in the delivery of cargo-enriched transport carriers to the plasma membrane and the TGN.

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Declaration of interest

The authors declare no competing financial interests

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Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

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Annotated references:

[7] * A recent review of the recently identified cargo selective complexes that localise on endosomes and interact with the WASH complex to coordinate the retrieval and recycling of cargoes.

[14] ** This elegant paper not only identifies retriever, a novel retrieving complex, but also shows that the recruitment of WASH complex is not solely dependent on the association with the retromer complex.

[29] * This paper explores the role of actin in the maturation of the vacuolar domain of the early endosome where intraluminal vesicles are formed.

[41] ** A recent review on the WASP family members with the most up-to date discussion of the WASH complex assembly and function.

[42] * This paper implies that the different subunits of WASH complex might have specific functions in the process of cargo retrieval and recycling.

[47] ** This study establishes that the actin network can directly bind to cargoes and that this interaction is sufficient to overcome ubiquitin-mediated sorting in the ESCRT subdomain. This observation points towards a model whereby the branched actin network on endosomes could, directly or indirectly, restrict the lateral mobility of cargos preventing their sorting into the degradative subdomain.

[49] ** The work presented in this paper adds a novel layer to the complexity of the regulation of the WASH complex and establishes that the fine-tuned regulation of F-actin deposition is critical for the process of cargo recycling.

[54] ** This study reports that PI(3,5)P₂ controls the interaction between cortactin and actin and suggests an elegant model for the integration of the WASH and cortactin function based on the turnover of different phosphoinositides species on endosomes.

[69] ** This study establishes that the β_2 adrenergic receptor (β_2 AR) activates G α stimulatory protein (G α s) on the actin-rich endosomal tubules and not in the actin-depleted bulk recycling tubules. This suggest that regulation of actin dynamics on endosomes could tune downstream responses of GPCR signaling.

[70] * This paper identifies filamin A as a novel factor that regulates the dynamics of actin for the tubular-based recycling of GPCRs.

[75] * This work identifies the small GTPase RAB-10 and its effector EHBP-1 as novel factors that control endosomal recycling by bridging endosomal tubules to the actin cytoskeleton.

[77] * This paper discusses a novel, CYK-1/formin-dependent, mechanism that regulates actin dynamics for the endocytic recycling

[81] ** This study revealed that endosomal tubules create contact sites with the endoplasmic reticulum through a complex between the endosomal sorting nexin SNX2 and the ER tethered VAPs. The contact site functions as a platform for the turnover of endosomal PI4P whose levels are critical for the regulation of WASH-dependent actin nucleation.

[83] * The paper identifies a mechanism that is required for the generation of recycling tubules and that requires the integration of the microtubule-dependent pulling force with the actin-dependent stabilization of membrane tubules.

[84] * This work establishes that myosin VI couples to branched actin networks to promote the severing of the tubular carriers that are generated on melanosomes.

[89] * The work presented in this paper shows that Myosin VI localize to APPL1- and RAB5-positive signalling endosomes and mediate association of these compartments with cortical actin filaments. Importantly, this positioning of endosomes at the cell periphery plays a crucial role in the activation of AKT in response to extracellular stimuli.

Figure captions

Figure 1. Endosomal sorting of receptors

Transmembrane proteins that are fated for degradation, such as the activated epidermal growth factor receptor (EGFR), are initially subjected to ubiquitylation. Ubiquitin serves as a signal to sort the ubiquitylated cargo from the limiting membrane of the endosome into regions that invaginate and pinch off into the lumen of the endosomal vacuole to form cargo-enriched intraluminal vesicles (ILVs) [94]. The most important players in the sorting of ubiquitylated cargo into the forming ILVs are the endosomal sorting complex required for transport (ESCRT): ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III. ESCRT-0, -I and -II act to recognize the cohort of ubiquitylated cargoes while ESCRT-III is involved in the process of ILV biogenesis [94]. Through iterative rounds of cargo sorting and ILV biogenesis, the mature ILV laden late endosome becomes competent to fuse with the lysosome, leading to the formation of a hybrid organelle termed the endo-lysosome. Here the cargo present within the ILVs are degraded [95].

Alongside cargo sorting into ILVs, cargoes destined for recycling are sorted from the endosomal limiting membrane into branched tubular profiles, from where they are packaged in tubulo-vesicular carriers for transport to the cell surface or the biosynthetic pathway [2]. Cargo proteins that undergo endosome-to-*trans* Golgi network (TGN) recycling, this is given the specific term “retrograde transport”, include TGN-resident proteins that have reached the endosomal system through anterograde traffic including the sorting receptors that deliver the lysosomal hydrolases (such as the CI-MPR) [5]. Delivery of cargo back to the cell surface can occur directly, namely fast recycling, or indirectly, namely slow recycling, by means to transit through the endosomal recycling compartment (ERC) and possibly the TGN [6]. A subset of plasma membrane cargoes that include $\beta 1$ integrin (ITGB1) can take multiple recycling routes and might also undergo recycling through the TGN to be re-secreted in a controlled manner [96,97].

Historically, the mechanistic details of cargo recycling were considered to occur through sequence-independent “bulk” flow, as in the case of the Transferrin receptor (TfnR) [6]. However, more recent evidence is revealing that recycling of a multitude of cargoes, including the $\beta 2$ adrenergic receptor ($\beta 2AR$), is a highly regulated and sequence-dependent process that requires specialized endosomal sorting complexes that bind to retrieval and recycling motifs found in the cytosolic facing regions of functionally diverse cargoes [3]. It is now clear that several of these processes of cargo retrieval and recycling are regulated by actin.

Figure 2. Basis of sequence-dependent retrieval of cargoes in the actin-decorated subdomains.

The top panel illustrates the different multiprotein complexes known to play a role in the retrieval and recycling of cargo proteins on endosomes, the precise sequence motifs in the cytosolic domain of cargoes are reported between brackets. Retromer is an heterotrimer of the subunits VPS35, VPS29 and VPS26, that, directly or indirectly (via association with SNX3 or SNX27) interacts with different retrieval and recycling motifs of cargo proteins [3]. Importantly, the membrane remodelling SNX1/2:SNX5/6 complex, which was historically thought to be strictly connected to the retromer, has recently been shown to participate in the retromer-independent recycling of the receptors CI-MPR and IGF1R [17,18]. The multimeric CCC complex, which consist of the subunits CCDC22, CCDC93 and COMMD proteins, contributes to the process of cargo recycling [7,14,66]. The COMMD proteins are a family of ten highly conserved factors which seem to act as cargo adaptors with COMMD1 recently been shown to bind the receptor ATP7A [7,14,66]. Importantly the CCC complex is integrated into the retrieval subdomain via the interaction between CCDC22 and CCDC93 and the FAM21 tail [66]. Retriever is a stable retromer-like heterotrimer composed of the subunits: C16orf62, which is predicted to share structural similarities with VPS35 [14]; VPS29, which is shared with the retromer complex and DSCR3, which is a paralogue of VPS26. Like retromer coupling to the SNX27 cargo adaptor, retriever also binds to a cargo adaptor, in this case sorting nexin 17 (SNX17) [14]. Retriever associates both with the CCC complex and the WASH complex [14].

The bottom panel illustrates how the WASH complex, the major Arp2/3 activator that localizes at the surface of endosomes, interacts with all the cargo selective complexes and contributes to the architecture of the retrieval subdomain [3,7].

Figure 3. Phosphoinositides role in the remodeling of branched actin networks by the WASH complex and cortactin

The figure illustrates the possible interplay between phosphoinositides (PIs) together with the WASH complex and cortactin in regulating the dynamics of branched actin networks. PIs are lipids that are inserted in the membranes via their diacylglycerol “tail” while the inositol “head” group is exposed to the cytosol. The hydroxy residues present on the D3, D4 and D5 carbons of the inositol molecule can be phosphorylated, singularly or in combination, to generate one of seven phosphoinositides species found *in vivo* [98]. Different compartments of the cell are enriched with distinct PI species with early endosomes being enriched with PI3P whereas late endosomes are decorated with PI(3,5)P₂, PI4P and PI(4,5)P₂ [98]. PIs can be quickly generated, removed or inter-converted during specific cellular events and this flexibility is achieved via a number of PI kinases and PI phosphatases that can dynamically remove or conjugate the phosphate groups on the inositol head [98].

It is believed that: I) WASH associates on PI3P-positive endosomes through its interaction with retromer, which indirectly binds PI3P [43,45,46], with the ESCRT-0 component HRS [47], and possibly, on its ability to bind PIs [30,40]. II) Subsequently, the WASH complex induces the activation of the Arp2/3 complex and the generation of branched actin networks on endosomes. III) Cortactin is then recruited to nascent branch points where it further contributes to the assembly and stabilization of the actin networks [54]. IV) As the endosome matures, the kinase PIKfyve converts PI(3)P to PI(3,5)P₂ [56]. V) PI(3,5)P₂ sequesters cortactin, preventing the binding for actin filaments, and possibly releases the WASH complex [54]. In the absence of cortactin at the branchpoint the Arp2/3 complex loses affinity for the actin filament resulting in an overall disassembly of the branched actin networks.

It has recently been shown that VI) the ER-endosome contact sites regulate the endosomal levels of PI4P, the accumulation of which causes exaggerated WASH mediated activation of actin nucleation and formation of actin comets on endosomes [81].

Figure 4. Schematics of regulated recycling of receptors through actin-rich tubular domains.

The Figure illustrates the central role of actin dynamics in the sequence-dependent recycling of cargoes, from membrane deformation to the movements of cargo-enriched tubular profiles. The regulated sorting of receptors, contrary to the bulk flow, sequence-independent recycling pathway, is tightly regulated by actin dynamics at the surface of endosomes [52,67,69]. I) The retrieving machineries (see **Figure 2**) trap receptors with specific retrieval and recycling motifs in endosomal actin-enriched subdomains and allow the entry of the receptors in tubular profiles that are enriched with actin and actin-related proteins [52]. These tubular profiles are generated through a series of BAR (Bin/Amphiphysin/Rvs) domain-containing proteins including a number of BAR-domain containing sorting nexins (SNX-BARs) [99]. The SNX-BARs associate with endosomal membrane via a co-incidence detection of phosphoinositides via their PX domains, together with the sensing of membrane curvature via their BAR domains [100,101]. Upon localisation to membranes, it is thought that the increased concentration of BAR domain-containing proteins serves to promote their oligomerisation into higher order spiral arrays, which result in vesicle-to-tubule membrane remodelling [101]. Of these, the SNX-BAR proteins SNX1, SNX2, SNX5, SNX6 and SNX6b (also SNX32) are linked to the retromer pathway and coordinate the tubular-based recycling of the retromer retrieved cargoes [8]. The pulling force exercised by the microtubule motor dynein/dynactin complex contributes to the extension of the nascent carrier [102,103]. Moreover, the WASH complex interacts directly with tubulin, and thus it may assist the extension/stabilisation of tubular profiles on microtubule tracks [31]. II) Subsequently, the localised WASH-dependent nucleation of branched F-actin may contribute to the destabilisation of the tubular profiles by providing a pushing force to destabilise the membrane and induce membrane fission [30,31,46,65]. III) Lastly, the detached tubular carrier might use actin polymerization and actin-based motors to facilitate the short-range motility toward the final destination.

Figure 1

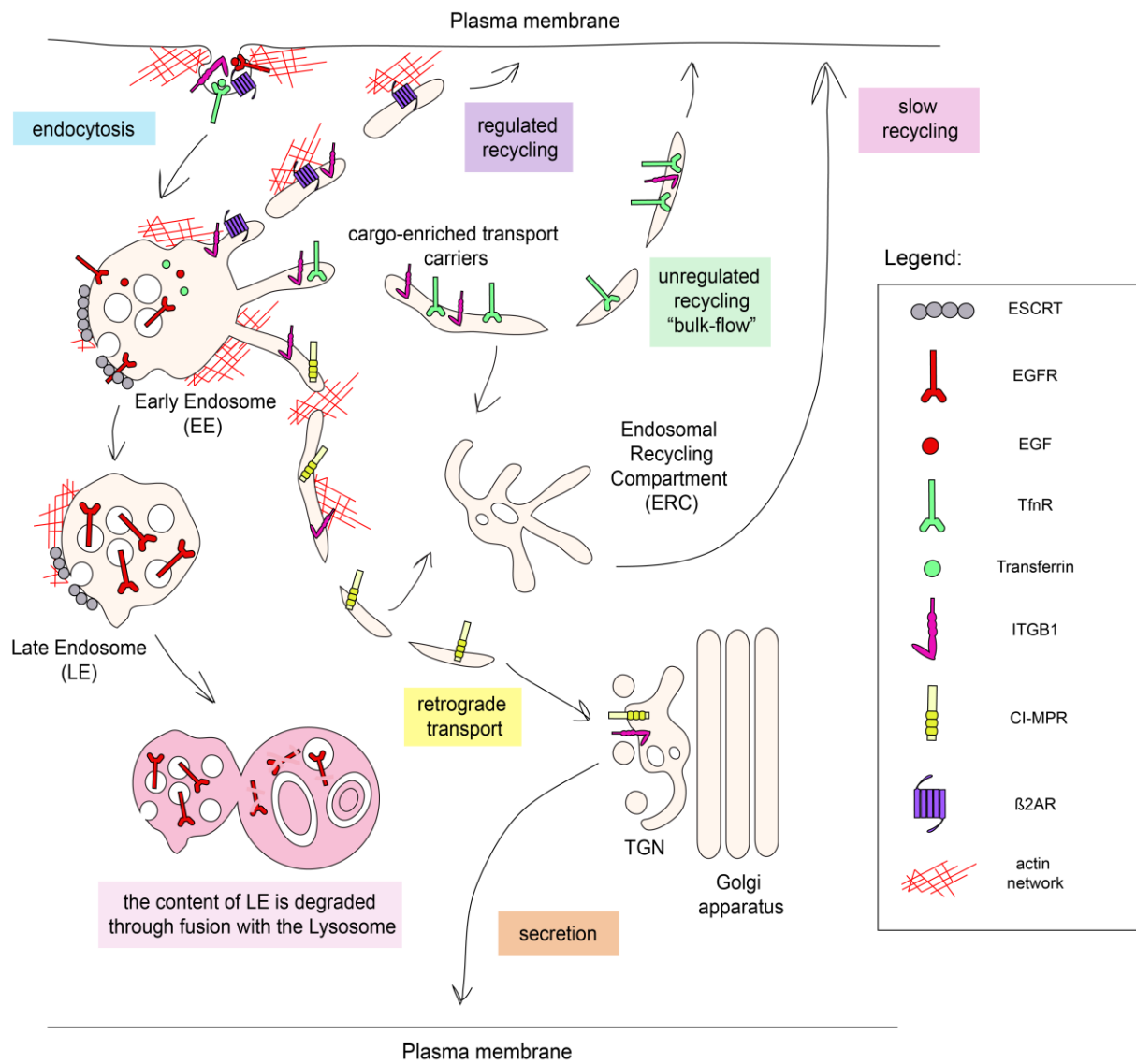


Figure 2

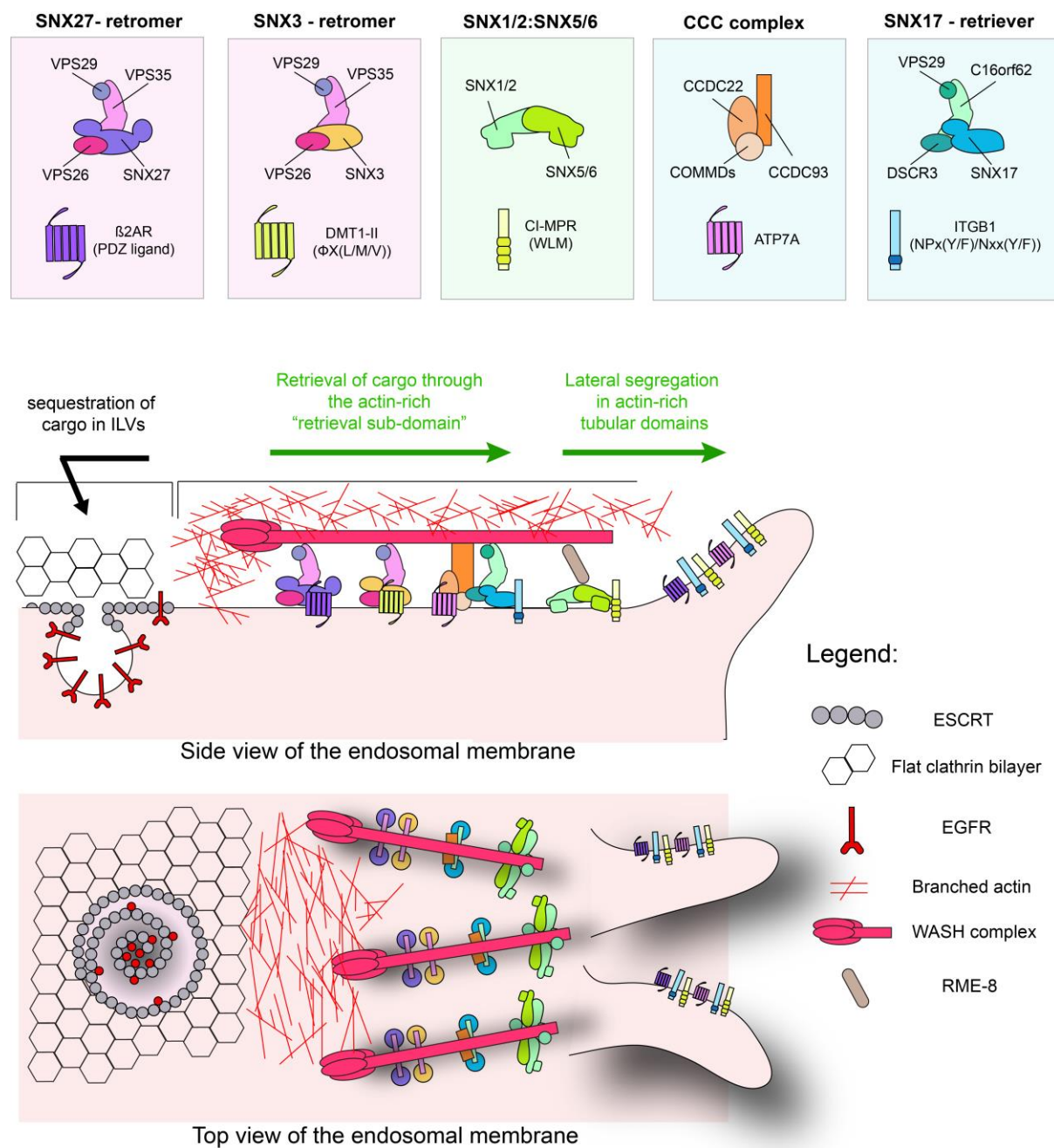


Figure 3

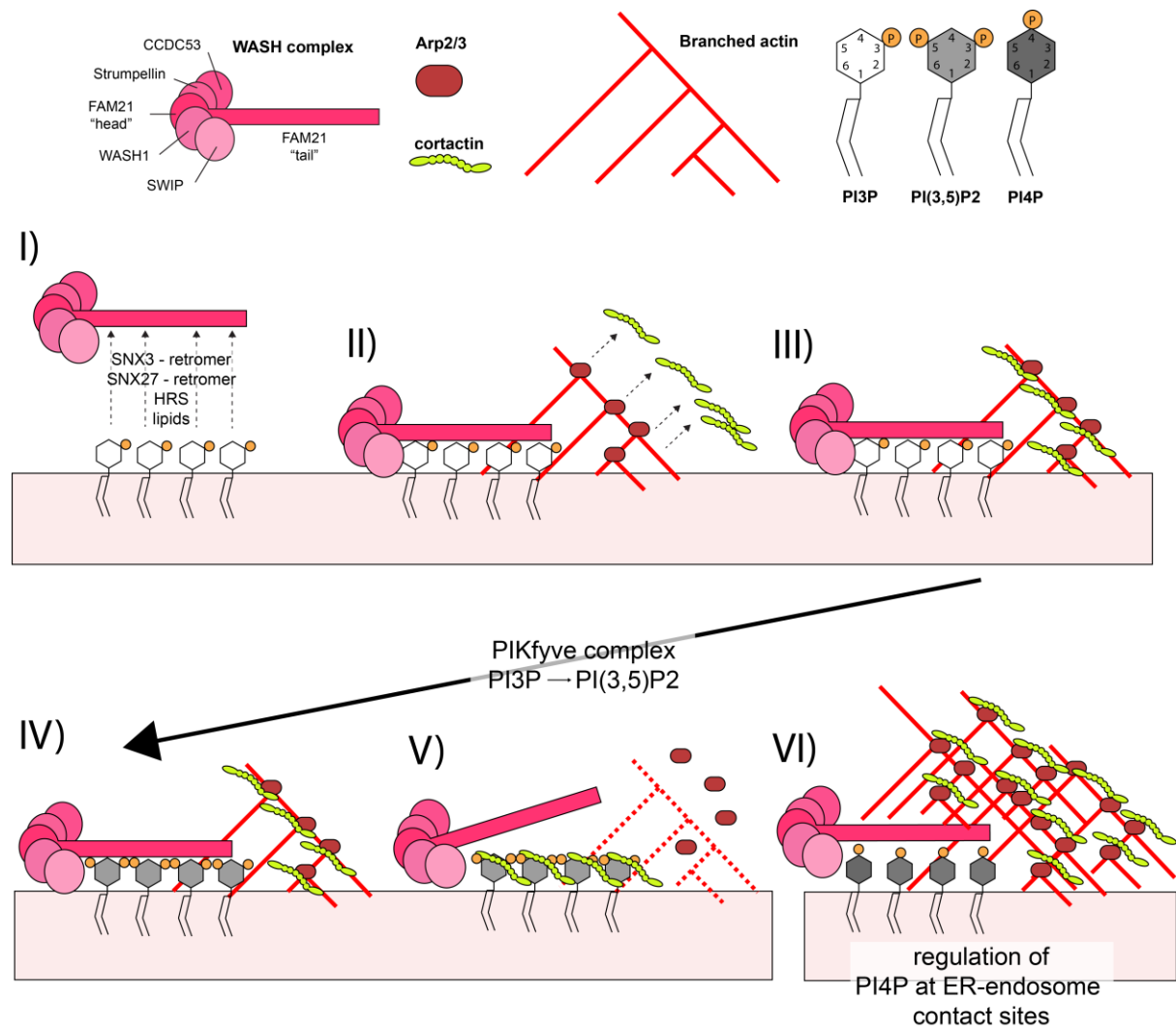
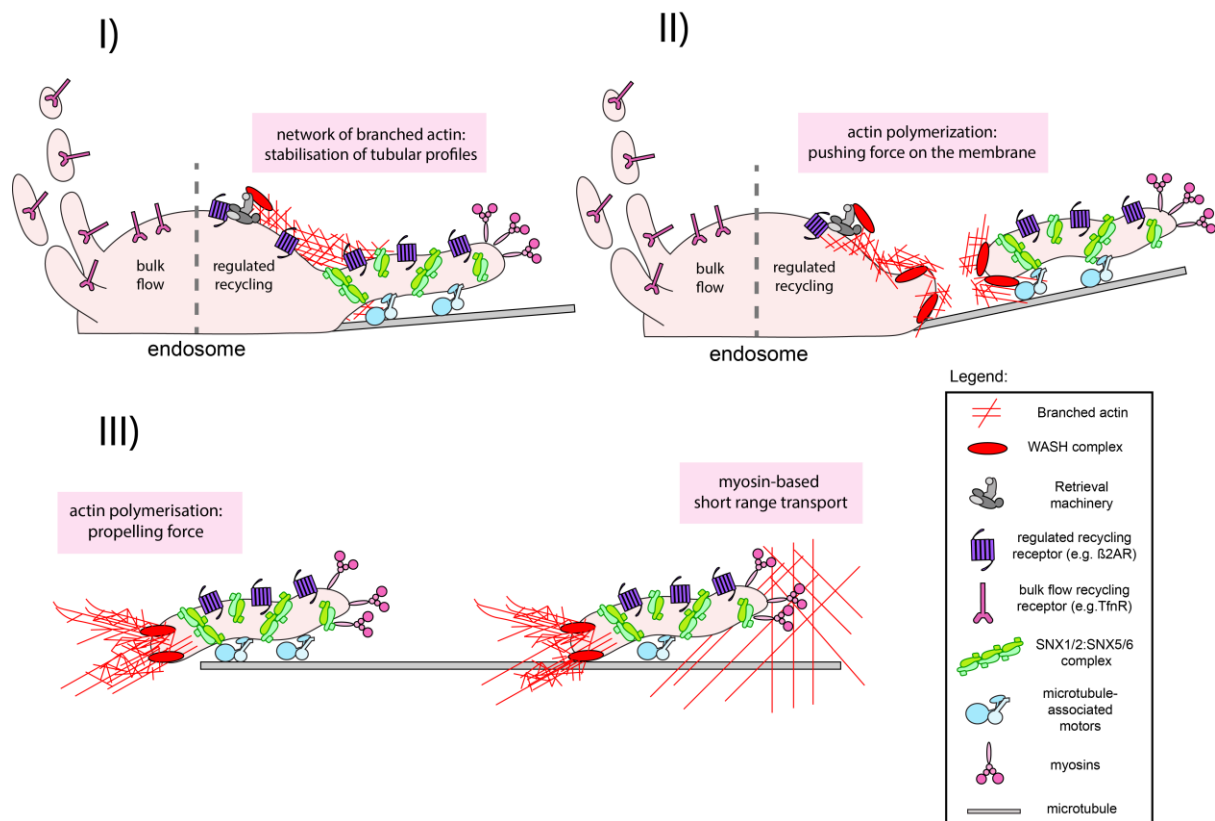


Figure 4



Graphical abstract

